Immunoglobulin G and Subclasses as Potential Biomarkers in Metastatic Melanoma Patients Starting Checkpoint Inhibitor Treatment

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Abstract: Checkpoint inhibitors have improved survival of metastatic melanoma. However, reliable biomarkers to predict response are still needed. Immunoglobulin G (IgG) antibody subclasses reflect immunocompetence in individuals and are known to be involved in essential functions in our immune system. This prospective study evaluated the association between serum IgG with its subclasses IgG1, IgG2, IgG3, and IgG4 and antitumor response according to RECIST 1.1. Serum samples from 49 patients were prospectively collected before the start of treatment with a checkpoint inhibitor. We observed a statistically significant association of baseline IgG2 with response to therapy (P=0.011). After defining optimal cutpoints, we found significant associations between total IgG (>9.66 g/L, P=0.038), IgG1 (>6.22 g/L, P=0.025), IgG2 (>2.42 g/L, P=0.019), and IgG3 (>0.21 g/L, P=0.034) with progression-free survival. Prolonged overall survival was associated with elevated IgG2 (>2.42 g/L, P=0.043). Together, these findings define total IgG and subclasses as predictors of clinical successful checkpoint inhibition in metastatic melanoma patients.

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Immunoglobulin G and Subclasses as Potential Biomarkers in Metastatic Melanoma Patients Starting Checkpoint Inhibitor Treatment

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Summary: Checkpoint inhibitors have improved survival of metastatic melanoma. However, reliable biomarkers to predict response are still needed. Immunoglobulin G (IgG) antibody subclasses reflect immunocompetence in individuals and are known to be involved in essential functions in our immune system. This prospective study evaluated the association between serum IgG with its subclasses IgG1, IgG2, IgG3, and IgG4 and antitumor response according to RECIST 1.1. Serum samples from 49 patients were prospectively collected before the start of treatment with a checkpoint inhibitor. We observed a statistically significant association of baseline IgG2 with response to therapy ($P = 0.011$). After defining optimal cutpoints, we found significant associations between total IgG (> 9.66 g/L, $P = 0.038$), IgG1 (> 6.22 g/L, $P = 0.025$), IgG2 (> 2.42 g/L, $P = 0.019$), and IgG3 (> 0.21 g/L, $P = 0.034$) with progression-free survival. Prolonged overall survival was associated with elevated IgG2 (> 2.42 g/L, $P = 0.043$). Together, these findings define total IgG and subclasses as predictors of clinical successful checkpoint inhibition in metastatic melanoma patients.

Key Words: immunoglobulins, subclasses, metastatic melanoma, checkpoint inhibitor, biomarker, response

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Checkpoint inhibitors (CIs) have revolutionized the treatment of several cancer types including melanoma, which is reflected in a significantly increased patient survival. CIs targeting the CTLA4-B7 (iplimumab) or PD1-PDL1 (nivolumab, pembrolizumab) axis have been approved for melanoma and show response rates around 40% with anti-PD1 monotherapy and 60% with the combination of ipilimumab and nivolumab.[1–6]

Melanoma is a rather immunogenic tumor. Spontaneous tumor regression has been reported on several occasions. Consistently, it shows a higher incidence in immunosuppressed patients.[7,8] This implies immunosurveillance of melanoma and likely explains the clinical effectiveness of immunomodulatory agents including CIs in metastatic melanoma.

Total serum immunoglobulin G (IgG) levels range from 7 to 16 g/L in normal individuals and consist of 4 IgG subclasses. IgG1 is quantitatively the most abundant IgG subclass known to be produced in response to soluble antigens and membrane proteins and functions to neutralize toxins and pathogens.[9] Immunoglobulin G2 (IgG2) is produced in response to bacterial capsular polysaccharide antigens and has the unique feature of not binding to Fc receptors on antigen-presenting cells. Immunoglobulin G3 (IgG3) is a potent proinflammatory antibody but has a low in vivo half-life explaining its relatively low abundance in serum.[10] Immunoglobulin G4 (IgG4) is thought to play an important role in allergic reactions together with immunoglobulin E (IgE). Unlike other IgG subclasses, it does not activate the complement system following antigen binding and is thus believed to act as an immune-regulatory IgG subclass. Consistently, during desensitizing immunotherapy to treat allergies, relief of symptoms correlates with the emergence of allergen-specific IgG4 antibodies.[11]

As IgG production requires functional antigen-presenting, T-helper and B cells, serum IgG subclass levels reflect the immunocompetence of an individual. Hypogammaglobulinemia, including selective IgG deficiencies, are known to result in susceptibility for infections and are also associated with autoimmunity.[12] As checkpoint inhibition requires a functional immune system, our hypothesis was that preexisting IgG and the composition of the IgG subclasses might be associated with efficacy of CI therapy in patients with metastatic melanoma. The aim of this prospective study was, therefore, to test whether pretreatment serum IgG and IgG subclass levels (IgG1, IgG2, IgG3, and IgG4) correlate with antitumor response and survival following CI therapy.

MATERIALS AND METHODS

Patient Cohort

We prospectively included patients with metastatic melanoma starting treatment with anti-PD1 or anti-CTLA4 antibodies at the Kantonsspital St. Gallen and University Hospital Zürich.

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Patients had at least 2 treatment cycles of either nivolumab (Opdivo; Bristol-Myers Squibb SA, 3 mg/kg every 2 weeks), pembrolizumab (Keytruda; MSD Merck Sharp & Dohme AG, 2 mg/kg every 3 weeks), ipilimumab (Yervoy; Bristol-Myers Squibb SA, 3 mg/kg every 3 weeks) or the combination of nivolumab and ipilimumab (1 and 3 mg/kg every 3 weeks). Serum samples were collected at the day of treatment start. Computed tomographic scans (CT) were performed after 10–12 weeks, and response was assessed according to RECIST 1.1 criteria.[13] Patients with progressive disease (PD) at the first CT scan were confirmed with another scan after 4–6 weeks with focus on potential pseudoprogression.[14] Responders were defined as complete remission (CR) or partial remission (PR). Nonresponders were defined as stable disease (SD) or PD.

The study was approved by the local ethics committees (EKOS 16/079, respectively, EK 647, EK800) and was carried out in accordance with the declaration of Helsinki principles.

Analyses of Immunoglobulins

Immunoglobulin levels were assessed by commercially available immunoturbidimetric methods (Binding site, Birmingham, UK) using an SPA plus analyzer (Binding site, Birmingham, UK). IgG subgroup concentrations (ie, IgG1, IgG2, IgG3, and IgG4) were determined using a Behring nephelometer II (BNII) (Siemens Diagnostics, Zurich, Switzerland) using reagents from Siemens (Siemens Diagnostics, Zurich, Switzerland). In our hands, the imprecision of the utilized methods, as assessed by coefficient of variations (CV) obtained from serial measurements of commercially available control materials, was as follows: 3% for total IgG (at concentrations of 7.1 and 13.2 g/L). The respective CVs for the IgG subgroups were as follows: 4.0% (at a concentration of 4.63 g/L) and 2.76% (at a concentration of 8.42%) for IgG1, 4.5% (at concentrations of 2.22 and 4.06 g/L) for IgG2, 6.17% (at a concentration of 0.22 g/L) and 5.07% (at a concentration of 0.4 g/L) for IgG3, and 5.7% (at concentrations of 0.38 and 0.96 g/L) for IgG4.

Statistical Analyses

R software (version 3.5.0) was used for all statistical analyses. The "survival" and "survminer" packages were used for survival analysis.[15,16]

The package "maxstat" was implemented to identify optimal cutpoints for biomarker levels that correspond to the most significant relation with progression-free survival (PFS) and overall survival (OS), using the maximally selected log-rank statistic.[17] The minimal proportion ("minprop" argument) in each group was set to 0.30. Kaplan-Meier plots were generated for OS and PFS, and the patients were categorized into "high" and "low" groups on the basis of the optimal cutpoint for continuous levels of total IgG and IgG subclasses. Hazard ratios (HR) for each biomarker were calculated using Cox's proportional hazards model. The association between IgG levels and PFS or OS was examined using the log-rank test.

RESULTS

A total of 49 patients were enrolled into the study. A total of 42 (86%) patients received monotherapy with an anti-PD1 antibody (nivolumab or pembrolizumab), 5 patients (10%) were treated with a combination of nivolumab plus ipilimumab, and 2 patients (4%) received an ipilimumab monotherapy.

One patient had a CR (2%) at the first CT scan, 23 had a PR (47%), 10 reached SD (20%), and 15 patients had PD (31%). Responders (CR, PR) versus nonresponders (SD, PD) were distributed as follows: 49% (n = 24) versus 51% (n = 25). Detailed patient characteristics are presented in Table 1. A total of 29 patients (59%) developed an adverse event of any grade (8 gastrointestinal, 8 endocrine, 7 skin, 3 renal, 2 pneumonitis, and 1 hematological).

IgG2 levels before initiation of CI therapy were significantly higher in the responder group versus the non-responder-group ($P = 0.011$). No significant differences were observed for total IgG, IgG1, IgG3, and IgG4 between responders and nonresponders (Fig. 1).

TABLE 1. Patient Characteristics

Age at start of immunotherapy (y)	
Median	68
Range	30–93
Sex [n (%)]	
Female	23 (47)
Male	26 (53)
Histology [n (%)]	
SSM	14 (29)
Nodular	17 (35)
LMM	1 (2)
Nevoid	1 (2)
Desmoplastic	1 (2)
Uveal	6 (12)
Mucosal	7 (14)
Amelanotic	2 (4)
Phototype [n (%)]	
1	1 (2)
2	26 (53)
3	13 (27)
4	1 (2)
NA	8 (16)
BRAF status [n (%)]	
Mutated*	13 (27)
Wild type	36 (73)
Type of immunotherapy [n (%)]	
Anti-PD1	42 (86)
Anti-CTLA4	2 (4)
Anti-PD1+anti-CTLA4	5 (10)
Metastatic sites [n (%)]	
1	11 (22)
2	16 (33)
3	7 (14)
4	6 (12)
5	5 (10)
6	2 (4)
7	1 (2)
8	1 (2)
Tumor response at first CT scan [n (%)]	
CR	1 (2)
PR	23 (47)
SD	10 (20)
PD	15 (31)
Treatment line [n (%)]	
First line	23 (47)
Second line	21 (43)
Third line	4 (8)
Fourth line	1 (2)

*V600 mutation.

Anti-CTLA4 indicates anticytotoxic-T-lymphocyte–associated protein-4; Anti-PD1, anti–programmed-cell-death protein-1; CR, complete remission; CT, computed tomography; LMM, lentigo maligna melanoma; NA, not applicable; PD, progressive disease; PR, partial remission; SD, stable disease; SSM, superficial spreading melanoma.

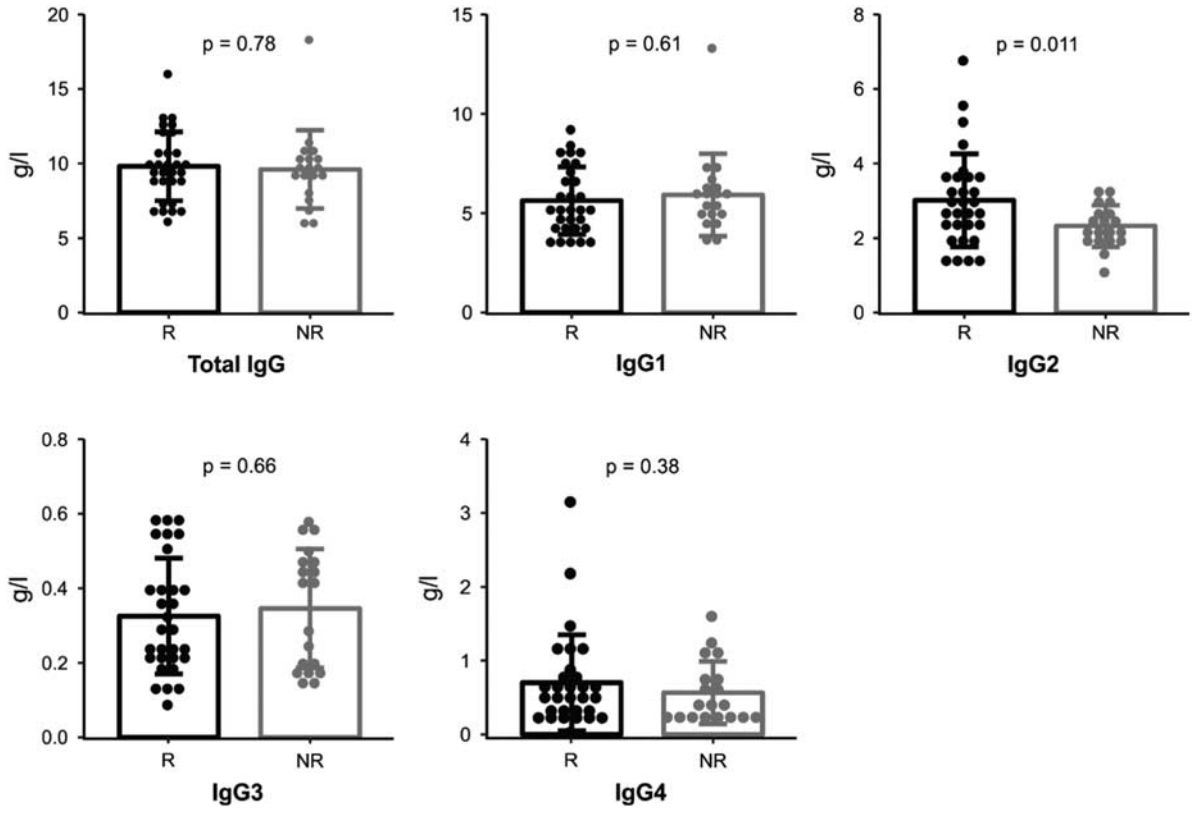

FIGURE 1. Baseline IgG and subclass levels before checkpoint inhibitor treatment in R versus NR. Reference values for total IgG and IgG subclasses in the white population of matching age to the subjects in this study are as follows: 7.00–16.00 g/L for total IgG; 4.05–10.11 g/L for IgG1; 1.69–7.86 g/L for IgG2; 0.11–0.85 g/L for IgG3; and 0.03–2.01 g/L for IgG4. IgG indicates immunoglobulin G; NR, nonresponder; R, responder.

Next, we evaluated whether specific levels of IgG and IgG subclasses were associated with PFS and OS. The median follow-up time was 20.7 months (interquartile range, 17.5–24.3 mo). For the calculated optimal cutpoints (see the Materials and methods section), PFS was significantly better in patients with high levels of total IgG [> 9.66 g/L, HR = 0.43, confidence interval: 0.18–0.98, $P = 0.038$], IgG1 (> 6.22 g/L, HR = 0.32, confidence interval: 0.11–0.92, $P = 0.025$), IgG2 (> 2.42 g/L, HR = 0.41, confidence interval: 0.19–0.88, $P = 0.019$), and IgG3 (> 0.21 g/L, HR = 0.45, confidence interval: 0.21–0.96, $P = 0.034$) (Fig. 2). Furthermore, OS was significantly prolonged in patients with IgG2 levels > 2.42 g/L (HR = 0.41, confidence interval: 0.17–1.00, $P = 0.043$) (Fig. 3). All the significant associations remained such after a false discovery rate of 0.10 was imposed using the Benjamini-Hochberg correction for multiple comparisons.

No statistically significant difference of IgG levels between responders and nonresponders (St. Gallen cohort) was seen during the course of treatment, and no statistically significant association was seen with adverse events.

DISCUSSION

To our knowledge, this is the first study providing evidence that baseline serum immunoglobulin levels may serve as predictive markers for response to CI therapy. Furthermore, a prognostic association of IgG subclass levels and survival was demonstrated. Until now, several baseline immune blood parameters were evaluated for their effect on response and survival for treatment with CIs in metastatic melanoma. Associations between better outcome and high lymphocyte count,[18–21] a low neutrophil count,[22] and a low neutrophil/lymphocyte ratio are known.[23] All these results underline the importance of T-cell activity against cancer cells. Our study focusing on the role of humoral immunity for CI therapy hypothesizes that B cells also may be involved.

Of note, measuring immunoglobulins in the serum is a simple, reliable, and rather cheap procedure readily available in virtually all routine medical laboratories.

Low serum IgG2 and IgG4, but not IgG3, have been recently shown to be associated with the clinical diagnosis of antibody deficiency in a retrospective analysis.[24] Thus, patients with low IgG2 and IgG4 may suffer from a mild form of immunodeficiency that was previously not recognized in these patients. It is interesting to note that, in 2013, Karagiannis et al[25] described an association between high IgG4 levels and poor survival in 57 patients diagnosed with stage I–IV malignant melanoma. However, this study did not include patients receiving CI therapy.

Low serum IgG levels may result from functional abnormalities of B cells but also T cells. This is exemplarily shown in human CTLA4 insufficiency, which is thought to be a T-cell intrinsic defect, associated with antibody deficiency in most patients.[26] Subsequent prospective studies are needed to test B and T-cell intrinsic functions in patients with normal or low IgG2 levels.

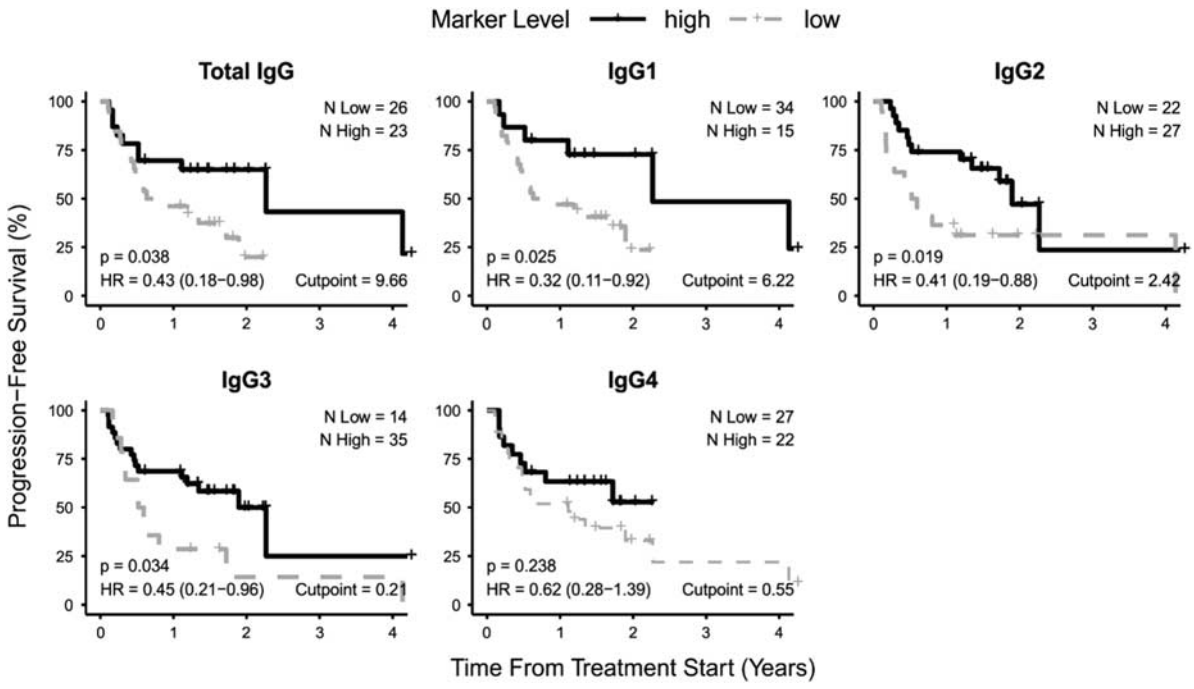

FIGURE 2. Progression-free survival depending on baseline serum IgG and IgG subclass levels (below and above cutpoint). HR indicates hazard ratio; IgG, immunoglobulin G.

Elevated IgG2 antibody levels in responders might reflect the presence of specific antibodies against melanoma self-antigens such as TRP1, TRP2, and gp100 or the cancer-testis antigen NY-ESO-1. The presence of these antibodies may, therefore, be a surrogate marker for inflamed tumors.

The following limitations have to be addressed. First, the relatively small number of patients does not allow us to perform extensive multivariable analyses. Nevertheless, our findings are statistically significant. Second, we do not have a validation cohort. However, the fact that our study is

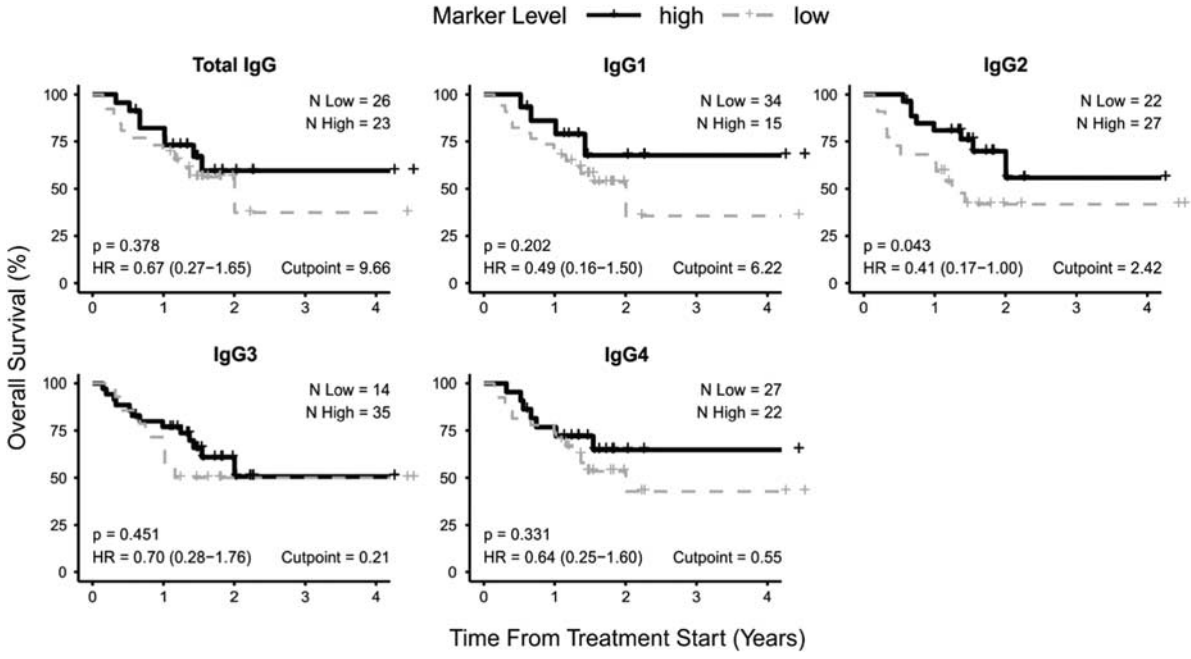

FIGURE 3. Overall survival depending on baseline serum IgG and IgG subclass levels (below and above cutpoint). HR indicates hazard ratio; IgG, immunoglobulin G.

prospective strengthens the hypothesis that these findings are generally applicable.

Furthermore, a certain number of uveal and mucosal melanoma patients was included, in which CI treatment is less effective with lower response rates.

In conclusion, serum total IgG and IgG subclass measurements at baseline may serve as biomarkers for checkpoint inhibitor efficacy in patients with metastatic melanoma. These findings are hypothesis generating and have to be confirmed in prospective studies with larger patient cohorts.

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Conflicts of Interest/Financial Disclosures

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All authors have declared that there are no financial conflicts of interest with regard to this work.

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